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RESEARCH ARTICLE

EXPRESSION OF B CELL POPULATION IN HASHIMOTO'S THYROIDITIS BY USING IMMUNOHISTOCHEMISTRY

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ABSTRACT

Background: Hashimoto's Thyroiditis is an established risk factor for development of lymphoma in thyroid and because of florid lymphocytic infiltrate it becomes difficult to distinguish it from lymphoma thereby posing a diagnostic challenge. The aim of the study was to demonstrate the demographical and morphological profiles of Hashimoto's thyroiditis and to ascertain the importance of B cell and T cell population using CD20 and CD 3 monoclonal antibodies for differentiating Hashimoto's Thyroiditis with extensive lymphoplasmacytoid infiltrate from MALT Lymphoma.

Materials and Methods: The study was conducted at the Meenakshi Medical College and Research Institute Hospital from September 2011 to July 2013. A total of 40 cases were studied. The specimen consisted of partial as well as total thyroidectomy specimens. Specimens were received in formalin and sections were processed and embedded in paraffin after gross examination. Haematoxylin and Eosin staining were done as routine in all the cases. Immunoreactivity with CD20 and CD3 was graded according to the scoring system and statistical analysis was carried out using SPSS version 19.0 software with regression modules installed.

Results: Correlation between cytological and histological diagnosis revealed autoimmune thyroiditis to be the most prevalent lesion followed by adenomatoid goitre with lymphoma to be least prevalent (1%). Immunohistochemical profile showed all cases (30) of Hashimoto's thyroiditis to be positive for CD20 and CD3 positive in 29 of the 30 cases, all cases of Adenomatoid goitre (9) were negative for CD20 with single case of Lymphoma showing intensely positive for CD20 and negative for CD3.

Conclusion: Strict morphological and immunohistochemical criteria are required to differentiate Hashimoto's thyroiditis from lymphoma. Cases with florid lymphocytic proliferation and any focus of atypical lymphocytes that masks earlylymphomatous transformation should be confirmed by CD20 & CD3 as well as Kappa and Lambda immunostaining.

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INTRODUCTION

Hashimoto's Thyroiditis is organ specific autoimmune disease characterised by diffuse goitre, hypothyroidism and production of anti-thyroid microsomal and anti-thyroglobulin antibodies. It is an established risk factor for development of lymphoma in thyroid gland. Because of the florid lymphocytic infiltrate it becomes difficult to distinguish from a lymphoma thereby posing a diagnostic challenge to the pathologists. It has a polyclonal cell population in the lymphoid follicles composed of B cells positive for CD45RO and CD3. It is a well known fact that almost all thyroid lymphomas arise in the setting of Hashimoto's thyroiditis which can cause development of mucosa associated lymphoid tissue (MALT) lymphoma which can ultimately lead to an aggressive lymphoma. There is a need to learn how to identify individuals who are at increased risk of developing lymphoma and how to intervene against this risk. This has led to the immunohistochemistry and molecular techniques to confirm or exclude the diagnosis of lymphoma. Thus the aim of the study is to identify B cell and T cell population in Hashimoto's Thyroiditis by means of immunohistochemistry using CD20 and CD3 monoclonal antibodies and to ascertain their usefulness in differentiating Hashimoto's Thyroiditis with extensive lymphoplasmacytoid infiltrate from MALT lymphoma.

Aims of the study

To demonstrate the demographical and morphological profiles of Hashimoto's thyroiditis and to ascertain the importance of B cell and T cell population using CD20 and CD 3 monoclonal antibodies.

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MATERIALS AND METHODS

The study was conducted at the Meenakshi Medical College and Research Institute Hospital from September 2011 to July 2013. A total of 40 cases were studied. The specimen consisted of partial as well as total thyroidectomy specimens. Specimens were received in formalin and sections were processed and embedded in paraffin after gross examination. Haematoxylin and Eosin staining were done as routine in all the cases. Immunoreactivity with CD20 and CD3 was graded according to the scoring system and statistical analysis was carried out using SPSS version 19.0 software with regression modules installed.

Inclusion criteria

Cases from september 2011 to July 2013 were included in the study.

Exclusion criteria

Cases showing toxic changes were excluded.

RESULTS

The study was conducted at the Meenakshi Medical College and Research Institute Hospital from September 2011 to July 2013 on a total of 40 cases. Of the 40 cases, Hashimoto's Thyroiditis followed by Adenomatoid Goitre and Lymphoma with the maximum occurring in the age group of 40-49 years. Female preponderance was found. Correlation between cytological and histological diagnosis revealed autoimmune thyroiditis to be the most prevalent lesion followed by adenomatoid goitre with lymphoma to be the least prevalent (1 %). Immunohistochemical profile showed all cases (30) of Hashimoto's thyroiditis to be positive for CD20 and CD3 positive in 29 of the 30 cases, all cases of Adenomatoid goitre (9) were negative for CD20 with single case of Lymphoma showing intensely positive for CD20 and negative for CD3.

Table 1. Distribution of Total No. of cases

S. No	Diagnosis	Number	Percentage (%)
1.	Hashimoto's Thyroiditis	30	75
2.	Adenomatoid Goitre	09	22
3.	Lymphoma	01	03

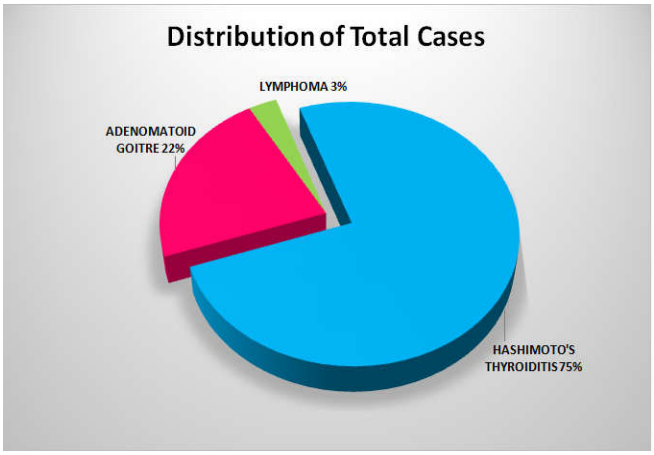

Figure 1. Distribution of total cases

Table 2. Sex wise distribution of lesions

S. No	Diagnosis	Male	Female
1.	Hashimoto's Thyroiditis	2(6.6)	28(93.4)
2.	Adenomatoid Goitre	3(33.3)	6(66.7)
3.	Lymphoma	1(100)	0(0)

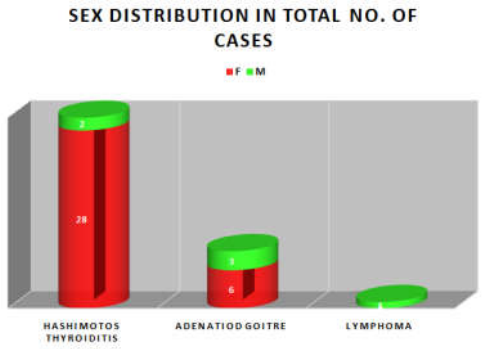

Figure 2. Sex distribution in total cases

Table 3. Correlation of Anatomical Diagnosis with Histopathological Diagnosis

S.No.	Anatomical Diagnosis	Histopathological Diagnosis	No. of Cases (%)	Total (%)
1	Diffuse	Hashimoto's Thyroiditis	22 (73.4)	26 (65)
		Adenomatoid Goiter	3 (33.3)	
		Lymphoma	1(100)	
2	Solitary	Hashimoto Thyroidits	7 (23.3)	11(27.5)
		Adenomatoid Goiter	4 (44.4)	
3	Multinodular	Hashimot Thyroidits	1 (3.3)	
		Adenomatoid Goiter	2 (22.3)	03 (7.5)
4	Total		40	40

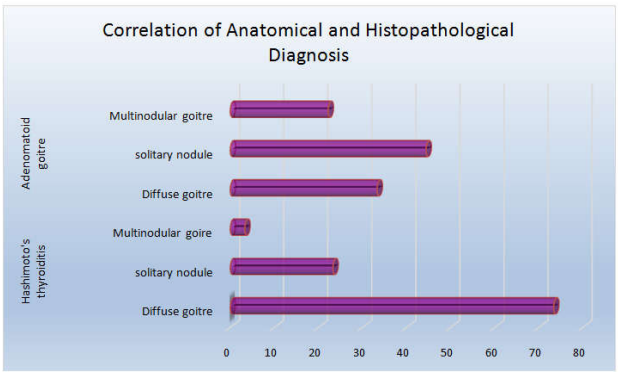

Figure 3. Anatomical and Histopathological correlation

Table 4. Correlation of Cytological Diagnosis with Histopathological Diagnosis

S. No	Cytological Diagnosis	Histopathological Diagnosis	No. of Cases (%)	Total
1.	Autoimmune Thyroiditis	Hashimoto's Thyroiditis	27(90)	27(67.5)
		Adenomatoid Goitre	00	
2.	Colloid Goitre	Hashimoto's Thyroiditis	1(3.3)	3(7.5)
		Adenomatoid Goitre	2(22.2)	
3.	Nodular Goitre	Hashimoto's Thyroiditis	1(3.3)	02(5)
		Adenomatoid Goitre	1(11.1)	
4.	Adenomatoid Goitre	Hashimoto's Thyroiditis	1(3.3)	07(17.5)
		Adenomatoid Goitre	6(66.7)	
5.	Lymphoma	Lymphoma	1(100)	1(2.5)
	TOTAL		40	40

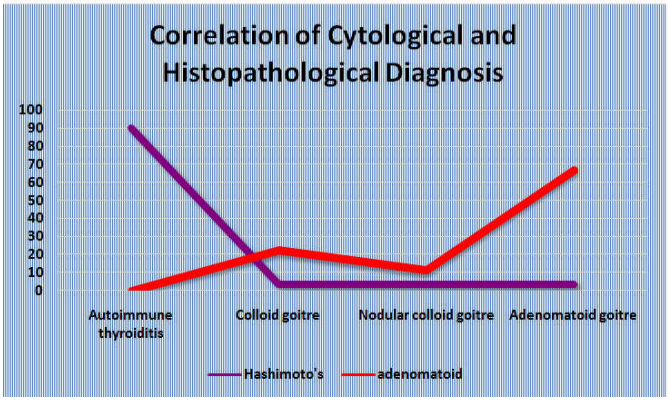

Figure 4. Cytological and Histopathological correlation

Table 5. Expression of CD20 and CD3 Positivity in total No. of cases

S.No.	Antibody	Histopathological Diagnosis	Positive	Negative	Total
1.	CD20	Hashimoto's Thyroiditis	30(100)	00	30
		Adenomatoid Goitre	00	09(100)	09
		Lymphoma	1(100)	00	1
	TOTAL		31	9	40
2.	CD3	Hashimoto's Thyroiditis	29(96.6)	1(3.4)	30
		Adenomatoid Goitre	00	09(100)	09
		Lymphoma	00	1(100)	1
	TOTAL		40		40

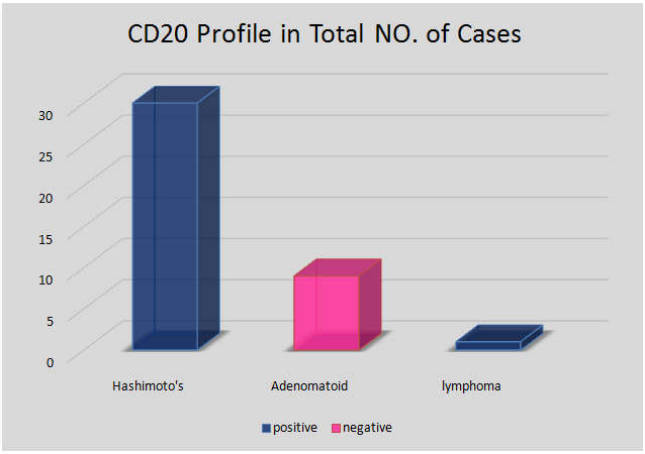

Figure 5. CD20 Profile in total cases

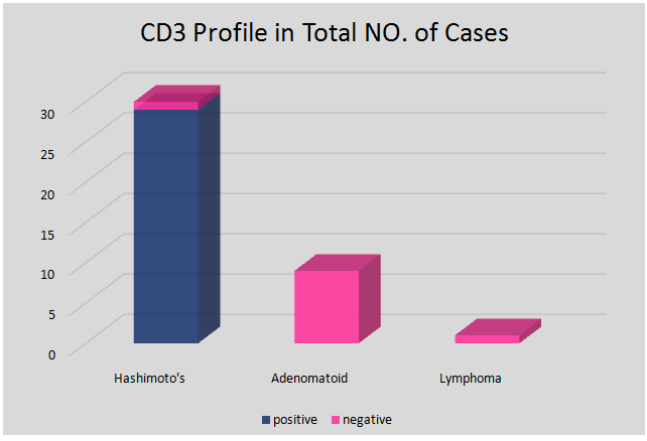

Figure 6. CD3 Profile in total cases

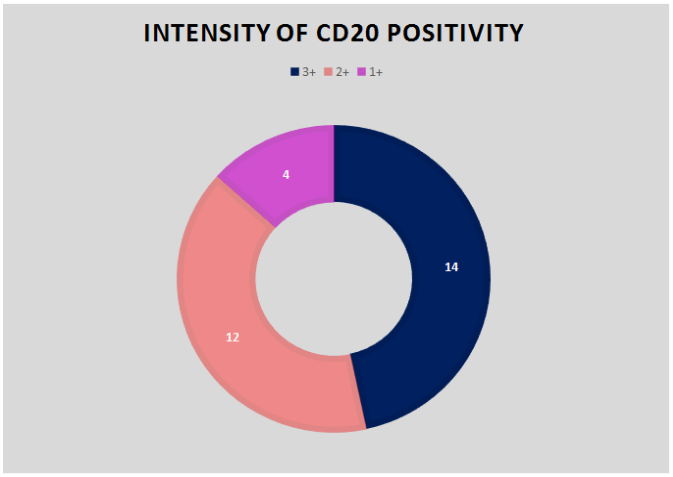

Figure 7. Intensity of CD20 Positivity

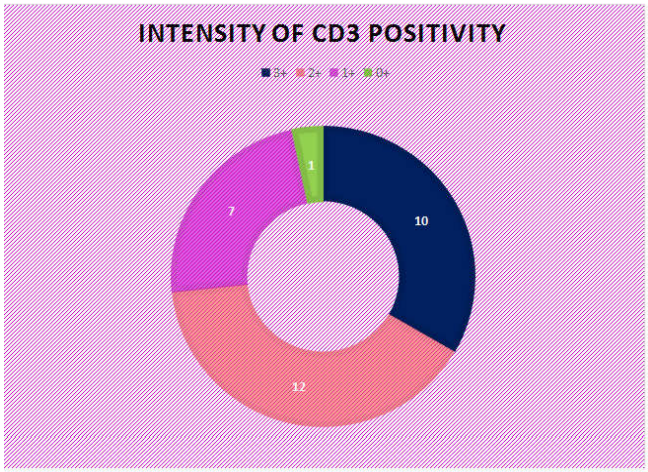

Figure 8. Intensity of CD3 Positivity

Gross pictures

Microscopy

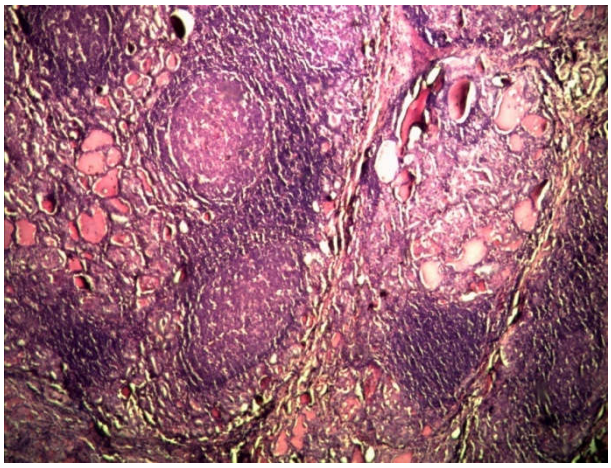

Figure 14. H & E (40X) Hashimoto's Thyroiditis

DISCUSSION

This study was conducted over a period of 2 years from September 2011 to July 2013 in the department of Pathology, Meenakshi Medical College Research and Institute Hospital. Prospective study of 40 cases with special emphasis on Immunohistochemistry using CD20 and CD3 was done with respect to age, sex, clinical presentation.

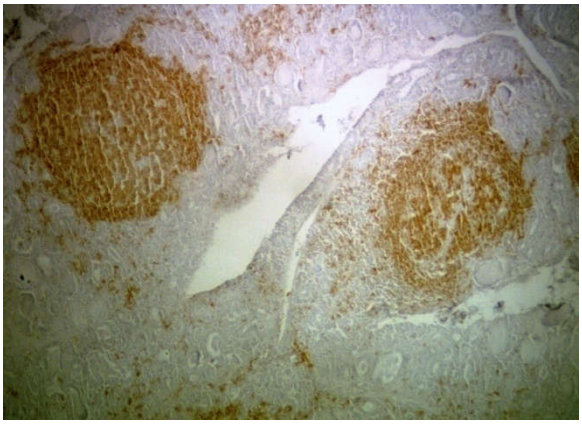

Figure 15. IHC(40x) CD20 Positivity in Hashimoto's Thyroiditis

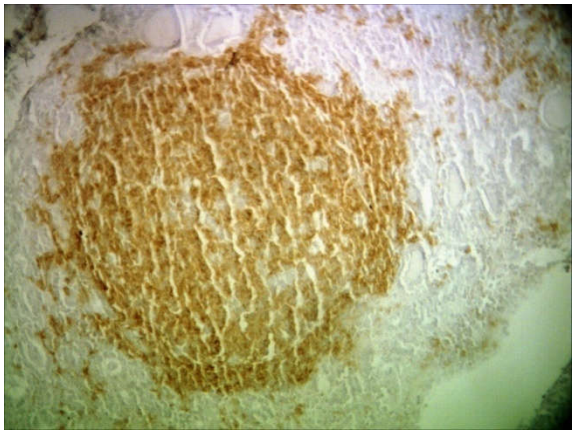

Figure 16. IHC(100X) CD20 Positivityin Hashimoto's Thyroiditis

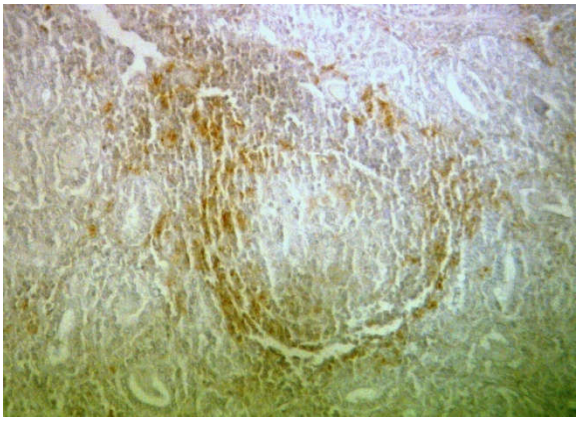

Figure 17. IHC(100x) CD3 Positivity in Hashimoto's Thyroiditis

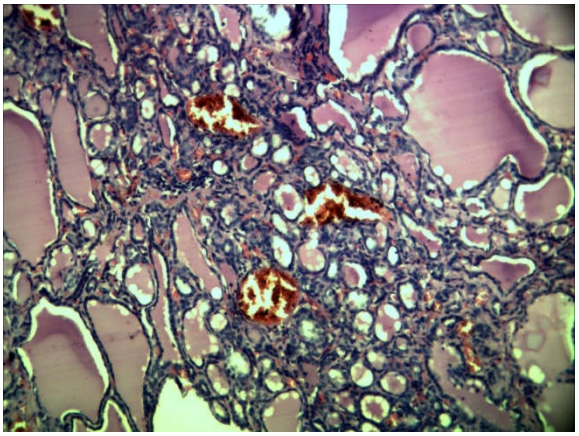

Figure 18. H & E(100x): Adenomatoid goitre

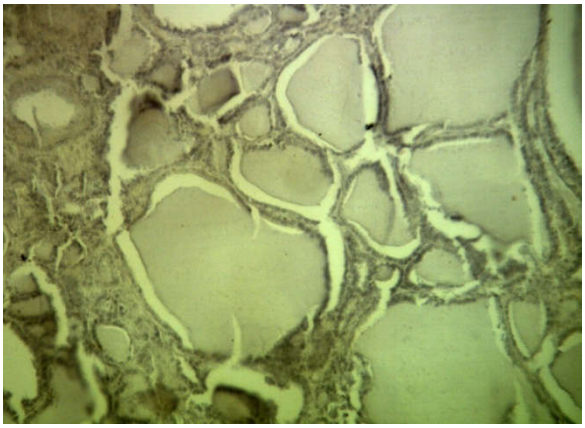

Figure 19. IHC(100x) CD20 Negativity in Adenomatoid goiter

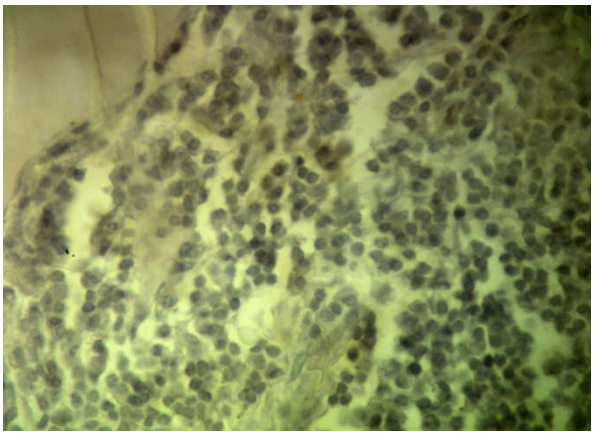

Figure 20. IHC(100x) CD3 Negativity in Adenomatoid goitre

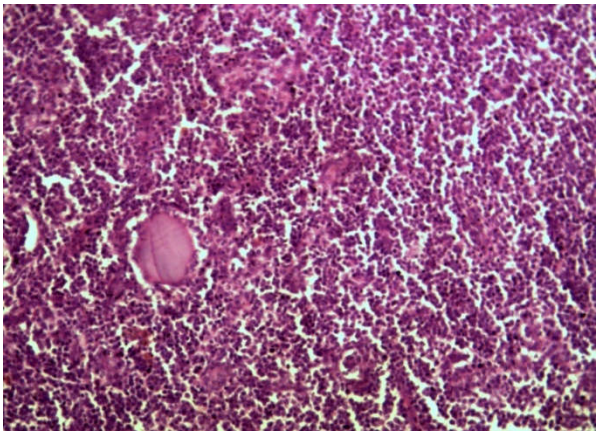

Figure 21. H & E(100x) Lymphoma showing effacement by monotonous population of lymphocytes

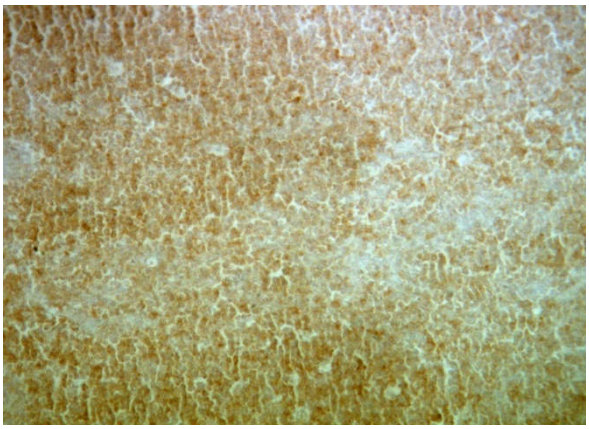

Figure 22. IHC(100x): Lymphoma showing CD20 Positivity

The various types of thyroiditis encompasses a heterogenous group of disorders ranging from acute bacterial to chronic autoimmune diseases. In clinical practice, inflammatory disease of the thyroid may be commonest of the thyroid abnormalities encountered.

Classification of thyroiditis (Singer, 1991)

1. Acute thyroiditis

2. Subacute thyroiditis
   a. Subacute granulomatous thyroiditis
   b. Subacute lymphocytic thyroiditis

3. Chronic thyroiditis
   a. Hashimoto's thyroiditis
   b. Riedel's thyroiditis

Hashimoto's thyroiditis can be graded pathologically as (Lakshman Rao and Reddy, 1991)

Grade I – Showing askanization of follicular cells and lymphocytic infiltration.

Grade II – Askanization of the follicular cells and lymphocytic infiltration with or without lymphoid follicle formation, destruction of follicles and varying degrees of necrosis.

Grade III– Extensive fibrosis in the gland with almost total disappearance of the follicle.

This is the stage of "burn out" disease.

In our study 40 patients were included, 30 of which were diagnosed as Hashimoto thyroiditis, 9 were diagnosed as adenomatoid goitre and 1 case diagnosed as lymphoma. Strong female preponderance was seen in concordance with the studies by Sharma *et al*, Fenn *et al* and Kazem *et al*. (Amani, 2011; Sharma *et al*., 1990; Fenn, 1980). In our study the age incidence ranged from 18 years to 58 years, youngest being a 18 year old girl and oldest being 58 year old woman. The mean age in study was 38.15 years with highest incidence being in between 40 -50 years. The mean age in Hashimoto's thyroiditis was 41.2 years. Kazem *et al* and Fenn *et al*. showed similar findings and was in concordance with our study (Sharma, 1990; Fenn, 1980). In our study majority of cases presented as diffuse goitre (65%) followed by solitary nodule (27.5%) and multinodular goitre (7.5%) which was similar to the findings in studies by Kazem *et al* and KusumKaplia *et al*. (Amani, 2011; Kapila *et al*., 1995). Histopathology of Hashimoto's thyroiditis revealed lymphoid infiltrate arranged in lymphoid follicles with interfollicular small round lymphocytes, plasma cells, scattered lymphoplasmacytoid cells and a few large transformed cells. Hurthle cell metaplasia were seen in almost all the cases. These findings were in concordance to the study conducted by Kazem *et al*. (Amani, 2011). Several studies have linked certain autoimmune and chronic inflammatory conditions to an increased occurrence of lymphoma. The magnitude of the average lymphoma risk in each disorder differs considerably among various studies. It was found that almost all thyroid lymphomas arised in the setting of Hashimoto's thyroiditis which induced reactive lymphoid proliferation that lead to the development of MALT lymphoma ultimately leading to aggressive lymphoma (Amani, 2011). The coexistence of reactive and neoplastic processes in the thyroid causes difficulty in diagnosing mucosa associated lymphoid tissue lymphoma (MALTOMA) using cytology and histology. This has led to the advancement of immunohistochemistry amd molecular techniques to confirm and exclude the diagnosis (Amani, 2011). We used immunohistochemical markers (CD20 and CD3) to evaluate B cell and T cell population in Hashimoto's thyroiditis. Our study showed 29 cases to be composed of small lymphocytes most of them are arranged as lymphoid follicles with CD20 positivity and CD3 negativity. The interfollicular lymphoid infiltrate were composed of T cells predominantly showing CD3 positivity and CD20 negativity. Our findings was in concordance with the studies conducted by Saxena *et al*. (2004) and Kazem *et al*. (Amani, 2011). All the cases of HT showed an admixture of B and T lymphocytes with CD20 highlighting the germinal centres while CD3 demonstrated the well developed mantle zone and the interfollicular population (His *et al*., 1998). Our study revealed one case of HT showing focal effacement of architecture with CD20 positivity and CD3 negativity thereby raising the possibility of harbouring a lymphomatous clone. D'Antonio *et al* also reported the existence of a minute focus of extranodal marginal zone lymphoma in case of HT thereby necessitating careful examination to disclose small foci of lymphomatous transformation (D'Antonio *et al*., 2009). Our study showed one case of thyroid lymphoma which was used as positive control. Microscopy showed total effacement of thyroid architecture by lymphoid infiltrates.

Conclusion

Hashimoto's thyroiditis is a common cause of goitrous enlargement of thyroid gland with hypothyroidism. It has a varied clinical presentation and can present as diffuse goitre, multinodular goitre or a solitary nodule. It is an established risk factor for the development of lymphoma but it differs both histopathologically and immunohistochemically from thyroid lymphoma and diagnosis is generally made by FNAC, antibody titre and histopathology. One case of thyroid lymphoma showed CD20 positive and CD3 negative thereby confirming B cell nature of the lymphoma. Clonal B cell proliferation in Hashimoto's thyroiditis has been detected by means of immunohistochemistry. One case showed CD20 positivity and CD3 negativity thereby raised the possibility of a clone being harboured. Kappa and Lambda immunostaining is required to demonstrate clonal expansion. Hence to differentiate Hashimoto's Thyroiditis from lymphoma strict morphological and immunohistochemical criteria has to be made. Cases of florid lymphocytic hyperplasia with focus showing atypical lymphocytes may mask early lymphomatous transformation thereby necessitating the use CD20 and CD3. Kappa and Lambda immunostaining contributes in demonstrating clonality and in ruling out the possibility of lymphoma arinsing in a background of Hashimoto's Thyroiditis. Strict morphological and immunohistochemical criteria are required to differentiate Hashimoto's thyroiditis from lymphoma. Cases with florid lymphocytic proliferation and any focus of atypical lymphocytes that masks earlylymphomatous transformationshould be confirmed by CD20 & CD3 as well as Kappa and Lambda immunostaining.

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